CHAPTER

7

Cu and Cu-based nanomaterials as nanofungicides

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1 Introduction

As the global demand for safe food production escalates against climate changes, it is clear that food safety and security is under great threat in modern-day civilization (Van Nguyen et al., 2020). This is exacerbated by fungal contamination with subsequent production of mycotoxins resulting in mycotoxicosis, which pose adverse harmful effects to human and animal health (Jarvis and Miller, 2005). Fungi are undesirable in food due to their degradation capacity and mycotoxins are linked to enormous economic losses as these organisms are always present in crops and grains, together with insects, and are the leading cause of stored product loss since insects tend to become spreaders of fungal spores. Conventional methods for agricultural protection against the ubiquitous fungal contamination have proven to be ineffective with high toxicity and onset of resistance attributed to fungicides (Singh et al., 2021a). New technological developments in farming techniques have used different fungicides to control fungal crop diseases and the search for new formulations is still required to circumvent the gradual increase in resistance and new strains of fungi.

We have records in history about the use of metals (e.g., copper, mercury, silver, and zinc) as disinfecting agents. During the period of the Ebers Papyrus, an Egyptian medical papyrus of herbal knowledge dating to circa 1550 BCE, utilized various copper-based constringents for treating infections (Arendsen et al., 2019). Furthermore, the Hippocratic Corpus, partially written by the great Greek physician Hippocrates, there is a mention of the use of copper-containing wound dressings and powders to treat leg ulcers and infection of fresh wounds. During the first century, in *De Medicina*, a medical writing by the Roman Aulus Cornelius Celsus, copper was regarded as an important drug to treat venereal diseases and nonhealing chronic ulcers (Walusinski, 2018). Copper was also used in the 19th century against the great European cholera epidemics. Greeks, Romans, Persians, Egyptians, and Indian ancient civilizations commonly used copper to purify water for consumption and food preservation (Nisar et al., 2019).

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The first modern use of copper in agriculture and food preservation for the control of fungi in crops dates back to 1761, when seed grains were soaked in a low-concentration copper sulfate solution; it was found to inhibit the seedborne fungi (Brauer et al., 2019). Later in the 1880s, a mixture of copper sulfate, lime, and water (Bordeaux mixture), and a mixture of copper sulfate (Burgundy mixture) were used as fungicides to spray grapes against mold, in the United States and France, respectively (Borkow and Gabbay, 2009). Since then, among the inorganic antimicrobial agents, copper compounds have been widely used in agricultural practices as a fungicide, pesticide, algaecide, herbicide, and also in livestock as disinfectants for animal husbandry purposes (Mastin and Rodgers, 2000).

Copper (Cu) is one of the most essential metal micronutrients required in very small quantities for proper protein function through coordination chemistry by plants and is used in many types of agricultural practices, particularly in organic agriculture (OA) (Chauhan et al., 2020; Da Costa et al., 2020; Malhotra et al., 2020; Rasool et al., 2021). Moreover, copper has been used as a biocide for centuries to disinfect solid surfaces, liquids, and human tissues (Borkow and Gabbay, 2004). Additionally, Cu functions as a cofactor for many enzymatic activities in plants involved in copper/zinc superoxide dismutase (Cu/ZnSOD), cytochrome-C oxidase, lysyl oxidase, tyrosinase, and dopamine hydroxylase, neuropeptide activation, connective tissue, lignin, and neurotransmitter synthesis, plastocyanin, and the ethylene receptors for the apoplastic oxidases (Da Costa et al., 2020; Lopez-Lima et al., 2021; Pilon et al., 2006). In addition, Cu is essential in plant respiration and metabolism of carbohydrates and proteins during photosynthesis, and seed production (Lopez-Lima et al., 2021; Pilon et al., 2006; Rasool et al., 2021; Yruela, 2005). Within the plant cell, Cu is required in at least six locations which include the cytosol, the endoplasmic reticulum (ER), the mitochondrial inner membrane, the chloroplast stroma, the thylakoid lumen, and the apoplast.

The main reason for the use of this metal is vastly attributed to its inherent antimicrobial properties; it was used even before the development of antibiotics to treat bacterial/fungal infections (Ingle et al., 2014). The antimicrobial properties of Cu can be exploited to control a variety of fungal and bacterial diseases (Quaranta et al., 2011; Weaver et al., 2010). Bulk Cu which is often supplied in ionic form (copper sulfate or copper hydroxide), exists in three oxidation states: Cu^0 (solid metal state); Cu^+ (cuprous ion); and Cu^{2+} (cupric ion) (Lopez-Lima et al., 2021; Malhotra et al., 2020). Cu^{2+} and Cu^+ have exhibited negative environmental effects on soil organisms (i.e., soil fauna and flora), and other symbiotic microbes such as fungal species (*Beauveria bassiana*) used as biocontrol agents against pests and insects and crop auxiliary species. This is attributed to repeated application of Cu-based pesticides resulting in Cu accumulation which oxidizes important biomolecules such as deoxyribonucleic acid, lipids, and proteins; and further exacerbated by molecular oxygen modification producing reactive oxygen species (ROS) through the Haber-Weiss and Fenton reactions, thus resulting in phytotoxicity (Hussain et al., 2021; Tegenaw et al., 2020).

Furthermore, Cu ions exhibit low stability in field conditions resulting in the formation of undesirable Cu complexes and high water solubility leading to chronic exposure to Cu which is inevitable, therefore, the recommended dietary intake of Cu for adolescents and adults is 900 μg/day which is primarily absorbed in the upper small intestine with 1/3 absorbed in the skeleton and muscle, with the median intake of Cu from food in the United States at approximately 1.0–1.6 mg/day for adult men and women (Malhotra et al., 2020; Pariona et al., 2019). The normal range of Cu in growth medium ranges between 0.05 and 0.5 ppm, with most plant tissues ranging between 3 and 10 ppm (Farid, 2015). In soil, the normal range for Cu is between 2 and 50 ppm (Da Costa et al., 2020; Rasool et al., 2021). On the other hand, in OA the use of synthetic fungicides is strictly prohibited, therefore; significant demand for alternatives is required. The objectives of this chapter are to provide an overview of (i) the use of Cu and Cu-based nanoparticles as nanofungicides in agriculture, (ii) green nanoagriculture approaches through Cu nanofungicides, (iii) provide insight on the Cu nanofungicidal mechanism of action, and (iv) highlight the ecotoxicology and safety of Cu nanofungicides.

2 Nanoagriculture

The use of nanotechnology in precision agriculture provides several benefits compared to traditional strategies in crop processes. Nanotechnology through nanocarriers have been used to deliver fertilizers, micronutrients, pest management, fungicides, herbicides, and biosensors (Chauhan et al., 2020; Duhan et al., 2017). Often methods used in agricultural areas, such as the application of chemical pesticides, has adverse effects on animals and are incredibly toxic and harmful to the environment (Damalas and Koutroubas, 2016). Their indiscriminate use increases pathogens and pest's resistance and reduces soil biodiversity by killing useful soil microbes. In addition to pesticide biomagnification, pollinators decline and destroy the natural habitat of farming, such as birds (Fischer et al., 2021; Rasool et al., 2021; Tilman et al., 2002).

There is a growing demand worldwide for food and continuous search for sustainable alternatives to increase the agricultural productivity efficiency without increasing planted area and negatively impacting the environment. Nanotechnological approaches have been proposed through several nonpersistent pesticide/fungicides formulations; including formulations based on nanomaterials for insect pests management, encapsulation of fertilizers for slow and controlled nutrients and water release, among other combinations (Bhan et al., 2018; Shang et al., 2019; Singh et al., 2021b). The use of nanotechnology through copper-based nanomaterials is a viable perspective that can effectively address various problems in crops. Copper or copper oxide nanoparticles (CuNPs and CuONPs, respectively) have excellent cytotoxic activity against a wide range of microorganisms: bacteria, fungi and viruses, including multidrug-resistant organisms (Chudobova et al., 2015; Ingle et al., 2014; Mahmoodi et al., 2018; Hai et al., 2021).

CuNPs and CuONPs present competitive advantages over other metals nanoparticles, such as silver, which also exhibit antimicrobial properties, as it is cheaper and easily available; therefore, the CuNPs/CuONPs are cost-effective (Camacho-Flores et al., 2015; Letchumanan et al., 2021). Nowadays, CuNPs and Cu alloys are also being used in many formulations and antimicrobial products such as synthetic textiles, food processing and packaging, biomedical and surgical devices, even in water purification. The reports available on antimicrobial studies of CuNPs prove its size-dependent efficacy against various pathogenic organisms in plants such as Gram-positive and Gram-negative bacteria, also to control yeast, and mold growth (Azam et al., 2012).

3 Green nanoagriculture

A recent trend in the field of nanomaterial science is "Green Nanotechnology," which is the use of nontoxic compounds for the synthesis and processing of nanomaterials in order to generate little impact on the environment for sustainably mitigating many challenges in the plant, human, and animal disease management (Singh et al., 2021a). Green nanotechnology provides a pronounced avenue for exploring the eco-friendly green synthesis for enhancing the properties of copper in a cost-effective manner, efficacious at low concentrations concomitantly reducing overt toxicity and resistance, and an additional armament of providing versatile scaffolds for the functionalization with other antimicrobial biomolecules from plants defense system (e.g., antiherbivory compounds, lectins, phytoanticipins, phytoalexins, and other defensive secondary metabolites) (Bartolucci et al., 2020; Benassai et al., 2021; Kanhed et al., 2014; Sanzari et al., 2019; Singh et al., 2021a). Green CuNPs and CuONPs can thus serve as an intervention for effective nanofungicides and growth stimulators in agriculture (Ermini and Voliani, 2021; Gámez-Espinosa et al., 2021; Malandrakis et al., 2021; Singh et al., 2021b).

Green nanotechnological approaches:

(i) Antimicrobial plant extracts and molecules used as reducing agents for the production of CuNPs and CuONPs providing a direct antagonistic effect against pathogenic fungi.
(ii) Surface modification of CuNPs and CuONPs with natural capacities of plants for resistance as plant defense stimulators to block mycotoxin production following fungal colonization.

One of such examples is demonstrated by Shiny et al. (2019), where they synthesized stable and environmentally benign CuONPs using various leaf extracts of Neem (Azadirachta indica), Pongamia (Pongamia pinnata), Lantana (Lantana camara), and extract of orange peel (Citrus reticulata). The plant extracts possess intrinsic preservative properties when coupled with biocidal properties of metallic nanoparticles such as Cu, can provide a synergistic effect as coating agents against fungi and termites, beneficial especially in timber farming and industry that produce primary wood products (e.g., furniture and rubber) and secondary products (e.g., wood pulp for the pulp and paper industry). Their results revealed that CuONPs synthesized with L. camara produced particles sized ranging from 33 to 46 nm, while the rest produced particles > 100 nm. The size difference is correlated to the effectiveness of L. camara CuONPs that inhibited 34.44% and 100% against the tested *Oligoporus placenta* (brown rot) and *Trametes hirsuta* (white rot) fungus, respectively. Moreover, L. camara CuONPs exhibited remarkable antitermite and rubber wood preservative activity when compared to the other CuONPs.

Ray et al. (2015) tested CuNPs synthesized by sugars and vitamin C against *Rhizoctonia solani* Kuhn fungal strain and observed a dose-dependent inhibitory effect with a maximum of 98.31% inhibition at 3.55 ppm with CuNPs synthesized in dextrose. Moreover, CuNPs synthesized in dextrin or in β-cyclodextrin had a slightly lower effect, but the peak of inhibition was achieved earlier. Interestingly, nanoparticles synthesized in cyclodextrin were up to 10 times bigger than their dextrin and dextrose counterparts, but the antifungal effect seemed not to be significantly dependent on size alone. The small dextrose-CuNPs, for instance, had a greater efficacy against the fungus compared to dextrin-CuNPs, probably due to a network structure formed by dextrose which enhances the interaction with the fungus (Ray et al., 2015).

Olchowik et al. (2017) evaluated the effect of CuNPs on the growth parameters of English oak (*Quercus robur* L.) seedlings using the foliar application. The CuNPs treatment on the degree of leaves infected by powdery mildew was also investigated. In general, the authors reported no phytotoxicity, and it was worth noting that the treatment did cause some ultrastructural changes in leaf chloroplasts but not in shoot and root tissues. However, effectiveness in controlling oak powdery mildew infection was not reported. Pariona et al. (2019) reported the use of ascorbic acid as both a reducing and stabilizing agent for the synthesis of CuNPs and evaluated their antifungal activity against *Fusarium solani*, *Neofusicoccum* sp., and *Fusarium oxysporum*. Interestingly, they showed using X-ray photoelectron spectroscopy (XPS) that the produced CuNPs' surfaces are populated with different Cu species (e.g., Cu^{2+}, Cu^{+}, and Cu^{0}) and were effective against the tested pathogenic fungi (Fig. 1).

Beltrán-Partida et al. (2019) demonstrated that CuNPs with around 250 nm diameter exert dose-dependent toxicity to Candida albicans strains isolated from oral denture candidiasis. A significant antifungal activity could be observed at 250 ppm, but the minimal inhibitory concentration was found to be 500 ppm. In this case, CuNPs require a longer incubation period compared to triclosan, a standard antifungal agent, but after 500 ppm their antifungal activity is similar. After 24 h, the researchers observed that the fungi were not forming pseudohyphae, most probably because of genetic alterations caused by Cu, and the permeability of plasma membrane and cell wall was impaired (Beltrán-Partida et al., 2019). The intense accumulation of CuNPs in the cytoplasm is able to trigger oxidative stress due to the formation of hydroxyl radicals by the contact between nanoparticles and membrane proteins, and the continuous action of those nanoparticles ultimately leads to cell lysis by oxidative stress and membrane dissociation. The authors

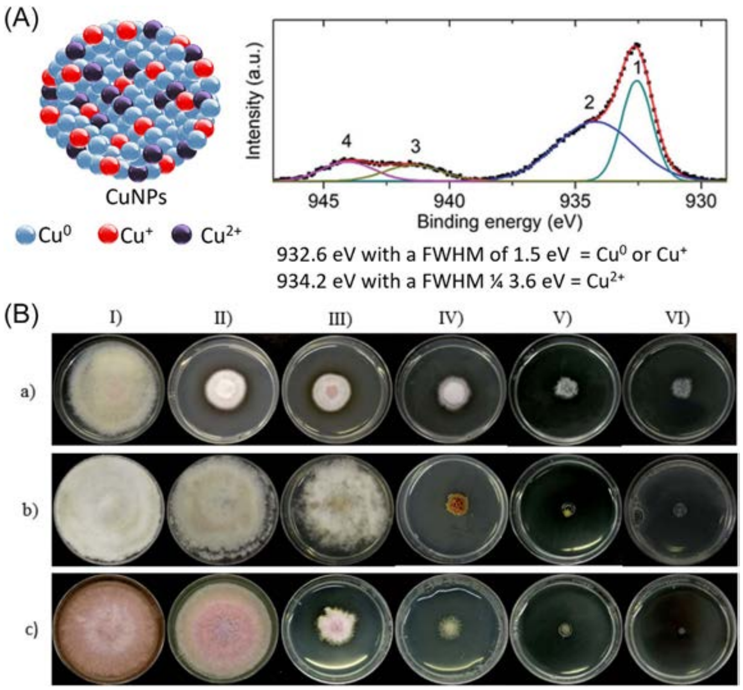

FIG. 1

Ascorbic acid CuNPs with differential Cu speciation—Cu^0, Cu$^+$, and Cu^{2+} (A) fitted XPS spectrum of CuNPs and (B) CuNPs antifungal activity against (a) *F. solani*, (b) *Neofusicoccum* sp. and (c) *F. oxysporum* at different concentration of CuNPs: (I) 0 (controls), (II) 0.1, (III) 0.25, (IV) 0.5, (V) 0.75, and (VI) 1.0 mg/mL of Cu-NPs.

Reproduced from Pariona, N., Mtz-Enriquez, A.I., Sánchez-Rangel, D., Carrión, G., Paraguay-Delgado, F., Rosas-Saito, G., 2019. Green-synthesized copper nanoparticles as a potential antifungal against plant pathogens. RSC Adv. 9, 18835–18843, with permission from the Royal Society of Chemistry under the Creative Commons Attribution License.

also observed that the presence of carbon and oxygen from ascorbic acid assisted in the antifungal effect of CuNPs (Beltrán-Partida et al., 2019). Wei et al. (2010) also verified that CuNPs oxidize and release Cu ions that are responsible for the oxidative stress that damages cell structures from the plasma membrane to intracellular organelles and biomolecules.

Finally, it has been demonstrated before that particles under 50 nm in diameter perform better than larger ones in terms of antimicrobial effects, especially if they are spherical (Safaei et al., 2019). Indeed, Ponmurugan et al. showed in 2016 that 2.5 ppm CuNPs (5–50 nm in diameter) reduced in 52.7% the incidence of *Poria hypolateritia*, compared to the 45.3% disease control achieved by bulk copper, although carbendazim drug performed slightly better (Ponmurugan et al., 2016). Table 1 summarizes the most important results regarding antimicrobial effects of CuNPs.

Table 1 Summarized results regarding the investigation of CuNPs antimicrobial effects.

Material characteristics	Size (nm)	Effective concentration against fungi	Summarized results	Ref.
CuNPs capped with polyvinyl-alcohol (PVA)	2–3	100% efficacy against *Corticium salmonicolor* with 7–10 ppm CuNPs	7 ppm of CuNPs were enough to inhibit the growth of *C. salmonicolor*, and spraying 10 ppm CuNPs was enough to kill most of the fungus that had already grown	Van Cao et al. (2014)
CuNPs synthesized by *Enterococcus faecalis* (extracellular method)	20–90	CuNPs (8 ppm) against *C. albicans;* 16 ppm *against Cryptococcus neoformans; and* 128 ppm for *Aspergillus niger* and *Fusarium oxysporum*	The efficacy of CuNPs was dependent on the fungus species, although the nanoparticles were more effective than regular antifungal drugs	Chavan and Kelmani (2014)
CuNPs dispersed in polymer films (PVMK, PVC, and PVDF)	~5	Excellent efficacy against *C. albicans* with 1 ppm	PVMK-CuNPs completely inhibited the yeast, while PVC- and PVDF-CuNPs were able to significantly inhibit fungal growth	Cioffi et al. (2005)
SiO-Cu and copper-doped hydroxyapatite nanopowder	3–15	14 ppm was effective against *C. albicans*	Hyperbranched polyamine/copper nanoparticles were effective against *C. albicans* even in low concentrations	Mahapatra and Karak (2009)
CuNPs stabilized with CTAB surfactant	5–10	20 µg per Kirby-Bauer standard paper disc presented good efficacy in inhibiting the growth of *Phoma destructiva, Curvularia lunata, Alternaria alternata,* and *F. oxysporum*	CuNPs provided better inhibition of the plant-infecting fungi compared to commercial fungicide, especially against *C. lunata* and *A. alternata*	Kanhed et al. (2014)
CuNPs in SDS micelles	50–70	A minimum inhibitory concentration of 3.75 ppm was achieved for *C. albicans* and *Candida parapsilosis*	CuNPs can be used as an antimicrobial agent for preventing biofilm growth and bacterial/fungal adhesion on medical materials	Kruk et al. (2015)
CuNPs stabilized with poly(acrylic acid)	15.6	Impregnation of wood with 3000 ppm was effective against *Trametes versicolor* and *Poria placenta*	Copper is a promising biocide agent for wood preservation. Its action was superior to silver in unleached wood exposed to *P. placenta*, but for leached wood, silver was superior	Pařil et al. (2017)
CuNPs stabilized with ascorbic acid	~250	Minimal Inhibitory Concentration of 500 ppm against *C. albicans*	CuNPs were able to decrease significantly the growth of *C. albicans* due to oxidative damage and cell content leakage	Beltrán-Partida et al. (2019)
Biosynthesized CuNPs	5–50	2.5 ppm of CuNPs significantly inhibited the growth of *Poria hypolateritia*	2.5 ppm could inhibit the disease in 52.7%, but Carbendazim had slightly better results (57.2% inhibition)	Ponmurugan et al. (2016)

Continued

Table 1 Summarized results regarding the investigation of CuNPs antimicrobial effects—cont'd

Material characteristics	Size (nm)	Effective concentration against fungi	Summarized results	Ref.
CuNPs synthesized in dextrose, dextrin or cyclodextrin in the presence of vitamin C.	Dextrose (6–8 nm), dextrin (5–6 nm) or cyclodextrin (50–80 nm)	3.5 ppm of CuNPs are effective against *R. solani*	The most effective CuNPs was dextrose-CuNPs (98.31% inhibition), followed by β-cyclodextrin-CuNPs (88% inhibition) and dextrin-CuNPs (84% inhibition)	Ray et al. (2015)
Alginate-CuO nanocomposite, synthesized under different conditions	37	4000 ppm of CuONPs with alginate were the most effective in inhibiting *A. niger*	Alginate-CuO nanocomposite inhibited 83.17% of *A. niger*, performing significantly better than bare CuONPs (57.24%)	Safaei et al. (2019)
CuNPs synthesized by chemical reduction (ascorbic acid)	20–50	*Fusarium* sp. were inhibited by CuNPs in a concentration-dependent manner (from 300 to 450 ppm)	The more CuNPs present in the culture medium, the smaller the fungal colonies, and the bigger the halo of inhibition. The best results were found for 450 ppm	Viet et al. (2016)
CuO nanoparticles	30	Foliar spray of CuONPs (500–1000 ppm) against *F. oxysporum* f. sp. *Niveum* to evaluate the effectiveness on suppressing *Fusarium* wilt and improving plant growth and yield	CuONPs suppressed *F. oxysporum* f. sp. *Niveum*. And yielded 39%–53% more fruits than untreated controls, moreover; polyphenol oxidase activity was elevated in plants treated with CuONPs. These results indicated that CuONPs may serve as effective delivery agent for Cu to suppress plant diseases	Elmer and White (2018)
nano-CuO synthesized extracellularly using *Streptomyces griseus*.	30–50	nano-CuO were sprayed on plants infected with *P. hypolateritia* (1.5 L/bush) and compared with treatments from bulk CuO and carbendazim. The soil and nutrient composition were also evaluated	nano-CuO increased TOC (3.5%), TN (3.6%), P (17.3%) and K (71.3%) in soil. Additionally, 2.5 ppm of CuONP—53% reduction in disease severity as compared to 45.3% and 57% reduction with bulk CuO and carbendazim, respectively	Ponmurugan et al. (2016)

CuNP, *copper nanoparticles;* CuONPs, *copper oxide nanoparticles;* PVMK, *polyvinylmethyl ketone;* PVC, *poly-(vinyl chloride);* PVDF, *polyvinylidenefluoride;* TOC, *total organic carbon;* TN, *total nitrogen;* P, *phosphorus;* K, *potassium.*

4 Mono- and/or hybrid copper nanomaterials as a fungicide

4.1 Copper nanoparticles (CuNPs)

The biosynthesis of CuNP powder using *Celastrus paniculatus* leaf extract and antifungal properties against *F. oxysporum* were explored by Mali et al. (2020). *F. oxysporum* was treated with CuNPs (0.12%, 0.18%, and 0.24%, *w/v* in water), $CuSO_4$ (0.1%, 1%), and plant extract. Results revealed that CuNPs showed a dose-dependent mycelia growth inhibition, with a maximum inhibition of 76.29 ± 1.52 at 0.24%, $CuSO_4$ with 20.74 ± 1, and the plant extract was not effective. This was accomplished by the flavonoid and other phenolic components in *C. paniculatus* leaf extract capping the CuNPs, resulting in a synergistic antifungal effect.

Lopez-Lima et al. (2021) demonstrated the beneficial bifunctional role of CuNPs as an effective nanofungicide and plant growth promoter against important soilborne pathogen *F. oxysporum* f. sp. *lycopersici* (FOL) that causes vascular wilt disease. Tomatoes (*Solanum lycopersicum* L.) are highly susceptible to FOL contamination. The CuNPs were produced using sodium citrate tribasic dihydrate and ascorbic acid (Pariona et al., 2019), and the antifungal activity of CuNPs and commercial copper hydroxide fungicide (Cupravit Hidro) against FOL growth was 67.3% and 15.6%, respectively, at 0.5 mg/mL. Moreover, CuNPs treatment diminished the symptoms of *Fusarium* wilt and increased the growth of tomato plants due to the increased chlorophyll content levels (from 19.3% to 28.6%) when compared to Cupravit Hidro. The authors deduced that the CuNPs effectively delivered Cu ions to the plant, while Cupravit Hidro facilitated uncontrolled Cu ion uptake, which hindered healthy plant development. Therefore, the CuNP treatment effectively provided a bifunctional approach for controlling *Fusarium* wilt and for promoting healthy tomato plant growth. The development of CuNPs/CuONPs fabricated with polymers and other metals provides enhanced antifungal properties and pathogen-induced defense mechanisms in plants for optimal protection.

Work by Van Nguyen et al. (2020) further exemplified the important role of Cu in agriculture. Seeds of the Vietnamese maize elite were treated with 4.44 ppm of CuNPs, the results suggested that CuNPs positively primed maize to regulate plant protective mechanisms associated with drought stress responses and tolerance through retaining 70% relative leaf water and enhanced superoxide dismutase (SOD) and ascorbate peroxidase (APX) enzyme activities, in addition to increased anthocyanin, chlorophyll, and carotenoid contents. Moreover, the treatment promoted maize growth, grain yield, and repression of ROS accumulation in maize under drought conditions for 21 days. This is of paramount importance in the midst of rising temperature surges attributed to global warming.

4.2 Copper nanocomposites (CuNCs)

The synergy between nanoparticulates and compounds that exhibit antimicrobial properties provide a benefit for the development of effective and enhanced fungicidal agents as hitherto incomprehensible approaches for agriculture through a myriad of biocompatible nanofungicides (Kamli et al., 2021; Malandrakis et al., 2021; Peixoto et al., 2020). One of the major hybrid copper nanocomposite involves copper-chitosan nanoparticles due to the collective synergy beneficial for the agricultural sector as a benign innovation (Bartolucci et al., 2020; Chauhan et al., 2020; Singh et al., 2021b). Abd-Elsalam et al. (2020) developed copper-chitosan nanocomposite-based chitosan hydrogels (Cu-Chit/NCs hydrogel) and evaluated their antifungal activity against aflatoxigenic strains of *A. flavus* (high producer (HP), intermediate producer (IP), and low producer (LP)) for aflatoxin B_1 and B_2. Results showed that Cu-Chit/NCs hydrogel antifungal activity at 240 ppm was effective for 100% fungal inhibition.

El-Abeid et al. (2020) developed a potent antifungal nanocomposite composed of reduced graphene oxide nanosheet-decorated with CuONPs—(rGO-CuONPs). The produced rGO-CuONPs (5, 20, and 50 nm) treatment was compared with conventional fungicide Kocide 2000 for their antifungal activity against *F. oxysporum* affecting tomato and pepper plants. Results revealed that rGO-CuONPs at 1 ppm showed the highest levels of fungal growth inhibition rate ($>85\%$) due to fungal cell membrane damage compared to Kocide 2000 which was only effective at 2500 ppm but only resulted in $<69\%$ fungal inhibition. Moreover, rGO-CuONPs drastically reduced *Fusarium* wilt and root rot diseases severity $<5\%$ for tomato and pepper plants with no phytotoxicity as shown in Fig. 2.

Most recently, Shang et al. (2020) produced copper sulfide nanoparticles (CuSNPs; ratio of 1:1 and 1:4 Cu:S) and investigated their nanofungicidal activity compared to commercial CuONPs and Kocide 3000 against pathogenic *Gibberella fujikuroi* in rice (*Oryza sativa* L.). The authors reasoned that since sulfur is involved in abiotic and biotic stress response through induced phytohormones, its incorporation with Cu will provide enhanced antimicrobial efficiency while simultaneously minimizing environmental toxicity. Results showed that 1:4 CuSNPs at 50 ppm has increased the growth of *G. fujikuroi* by 33% compared to CuONPs which caused inhibition by 18.7%. Furthermore, both 1:1 and 1:4 CuSNPs decreased disease incidence on rice by 35.1% and 45.9%, respectively, when compared to CuONPs at 8.1%, and Kocide 3000 exhibited no disease reduction. Additionally, 1:4 CuSNPs were found to stimulate critical phytohormones (salicylic acid and jasmonic acid) production to enhance pathogen-induced defense mechanisms against *G. fujikuroi* infection.

Xin et al. (2020) developed an innovative in situ system using polysuccinimide nanoparticles (PSI-NPs) capability of scavenging Cu from Cu contaminated soils as explained by to form Cu-PSI nanocomplexes in plants to reduce Cu^{2+} phytotoxicity. This was a result of amino acids (L-aspartic acid) of PSI-NPs that provided a negatively charged surface (-20.7 mV) allowing for the chelation of positively charged Cu^{2+} ions to form the Cu-PSI nanocomplex. The reported Cu-PSI nanocomplex served as a Cu sink for effective delivery and enhanced the adsorption of Cu in the plants due to the improved seed germination and seedling growth through enhanced antioxidative enzymatic activity, and increased water and Cu uptake.

The antimicrobial activities of Ag and Cu were exploited through the production of silver-copper nanocomposites (Ag/CuNCs) using *Olax scandens* leaf extract

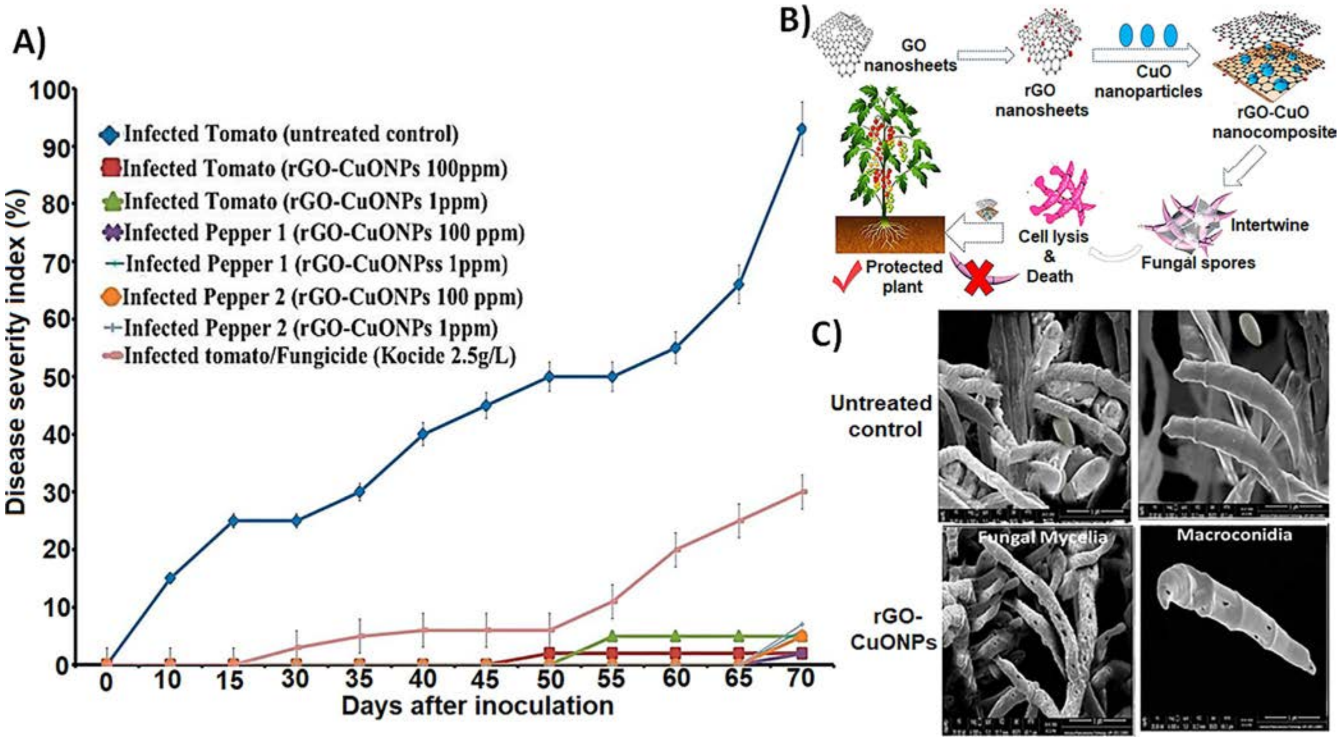

FIG. 2

Antifungal activity of rGO-CuONPs against *F. oxysporum* (A) disease severity index (%) of tomato and pepper plants with rGO-CuONPs and Kocide-3000 treatment for 70 days postinoculation; (B) schematic production of rGO-CuONPs and application for plant protection; (B) fungal inhibition growth rate of rGOCuONPs treatment; (C) SEM images of (a) untreated control and (b) rGOCuONPs interaction with fungal cells showing significant damage due to pores and irregular cavities on the cell wall surface.

Reproduced from El-Abeid, S.E., Ahmed, Y., Daròs, J.A., Mohamed, M.A., 2020. Reduced graphene oxide nanosheet-decorated copper oxide nanoparticles: a potent antifungal nanocomposite against fusarium root rot and wilt diseases of tomato and pepper plants. Nanomaterials 10, 1001. with permission from Nanomaterials under the Creative Commons Attribution License.

(Mujeeb et al., 2020). The Ag/CuNCs were composed of 33.35% Ag and 11.05% Cu to provide a bifunctional nanofungicidal activity against *F. moniliforme* and *C. albicans*. The data suggested that the minimal inhibition concentration of Ag/CuNCs was 125 ppm and 250 ppm for *F. moniliforme* and *C. albicans*, respectively. The antifungal activity was attributed to induced ROS generation by damaging cell membrane peptidoglycan, electron transport chain, protein synthesis, enzymatic degradation, and DNA damage; ultimately resulting in cell death (Hussain et al., 2021).

5 CuNPs/CuONPs nanofungicidal mechanism of action

With the emergence of pathogenic microorganisms resistant to antimicrobial drugs and an increased risk for nosocomial infections as a consequence, nanomaterials emerge as promising alternatives for pathogen control. Copper nanomaterials are already used as a fungicide, antibacterial and antifouling agents, but the mechanism of microbial growth inhibition is barely starting to be elucidated (Kruk et al., 2015). In the present day, among all metallic nanoparticles which present antimicrobial activity, silver has been the most intensively studied, and for many years the bactericidal and antifungal mechanisms of copper have been considered as the same as silver's (Ingle et al., 2014). Recent evidences show, however, that there are differences and similarities, but more studies must be performed in order to clarify the exact mechanism of CuNPs/CuONPs antifungal properties.

Copper is, indeed, known to be an effective biocide and is already available in pesticides and antifungal products, as it is capable of inhibiting the growth of various bacterial strains, algae, and fungi (Akturk et al., 2019) in a multifaceted approach but the exact mechanism is not yet fully understood. It has the advantage of being cheaper than silver and gold, therefore its use in antimicrobial products and strategies is constantly growing (Ingle et al., 2014; Van Cao et al., 2014). Besides the cost-effective property, CuNPs have the additional advantage of being easily oxidized to form more stable CuONPs, which can interact more intensively with cellular macromolecules (Ingle et al., 2014). The mechanism of action, most probably, relies on the dynamic structure of the plasma membrane from bacteria and fungi, which allow the permeation of CuNPs that release Cu^{2+} and Cu^{+} ions transported through the cell membrane via an energy-dependent transport system, thereby destabilizing the integrity of the membrane and trigger cell death pathways (Arendsen et al., 2019). Some lines of evidence also point to the toxicity of free metal ions that were dissolved from NPs attached to the plasma membrane, parallel to the oxidative stress caused by ROS generated on the nanoparticles surface (Akturk et al., 2019; Nisar et al., 2019).

In this regard, it has been observed that CuNPs can cause cell death by interacting with the DNA and disrupting the cell transcription and replication mechanism. Some proteins can lose their biological functions due to the interaction of CuNPs with their sulfhydryl groups, impairing important mechanisms (Nisar et al., 2019). Some authors hypothesized that CuNPs might exert some sort of effect on microbial plasma membranes as a consequence of copper affinity towards amines and carboxyl groups, which are often present in the cell membrane of bacteria and fungi. Furthermore, once inside the cells, copper may form crosslinks between the DNA strands, disturbing its helical structure (Ingle et al., 2014). Parameters such as particle size and stabilizing/capping agent are paramount for the efficacy of CuNPs in eliminating microbes (Akturk et al., 2019). It is well defined that some nanoparticles with different capping agents can lead to impairment in the antioxidant defenses and metabolic mechanisms within the targeted cells; therefore, the use of biocompatible capping agents is often strongly recommended. Those compounds can increase nanoparticles stability, half-life, cellular uptake, and reduced toxicity to the plant/crop being protected (Akturk et al., 2019). The concentration of nanoparticles arriving at the target tissue is equally important (Kalatehjari et al., 2015). Regarding the size role in antimicrobial properties of metallic nanoparticles, it is likely that the smaller the size (and consequently the higher surface area per unit of volume), the more intense is the interaction with the microbial membranes.

Pařil et al. (2017) observed that copper can improve wood decay resistance in leached and unleached wood, and it is known that the efficacy is significantly dependent on the size and surface chemistry of the nanoparticles, as well as their concentration. They also found that 3003 ppm of CuNPs was ideal for inhibiting the mass loss of leached and unleached wood exposed to white-rot fungus *Tinea versicolor* and red-rot fungus *Phormosoma Placenta*. Interestingly, silver nanoparticles (AgNPs) were less effective than CuNPs against *P. Placenta.* Furthermore, the researchers also observed that the efficiency of unleached wood decreased significantly after leaching. Chang et al. (2012) summarized the possible mechanisms of toxicity towards eukaryotic cells by copper and zinc nanoparticles. They theorized that those nanoparticles can enter the cells through pores or ion channels expressed naturally in the plasma membrane due to their small size, or via endocytosis, and once inside, they interact with cytoplasmic structures and some organelles, especially the mitochondria, generating an excess ROS which will damage various important biomolecules, such as DNA and metalloproteins (Chang et al., 2012).

5.1 Molecular mechanisms

Most of the knowledge regarding the antimicrobial properties of metallic nanoparticles comes from silver, and for a long time, it was accepted that the action mechanism of copper was mostly analogous. One of the most important effects caused by AgNPs in fungi is growth inhibition, followed by cell wall damage and impaired membrane stability. Furthermore, silver ions can react with different biomolecules, with a consequent loss of function: with DNA, it can lead to base complexes that suppress the replication of the genetic material; by reacting with thiol, carboxylate, phosphate, hydroxyl, amine, imidazole, and indole groups from enzymes, silver can inhibit important metabolic pathways and lead the cell to death; when interacting with the ribosome, through the downregulation of proteins and enzymes linked to ATP production (Mukherji et al., 2012). Silver also leads to the accumulation of phosphate, mannitol, succinate, glutamine, and proline in the interior of cells, interfering with the biochemical machinery and altering the cellular metabolism. All those effects might be due to the silver ions released in the surrounding aqueous environment, as similar findings can be observed after the administration of silver ions alone (Mukherji et al., 2012).

5.2 Copper nanoparticle-induced oxidative stress

Copper ions (Cu^{2+} and Cu^{+}) via oxidative reaction can be released from CuNPs and CuONPs, which are able to donate and accept electrons. This catalyzes the formation of ROS (hydroxyl radicals ($OH^{\bullet}$) and superoxide anions ($O_2^{\bullet-}$) that exert toxic effects on microbial cells via oxidative stress to membrane lipids (lipid peroxidation), amino acids in proteins, and nucleic acids that ultimately results in oxidative deactivation of enzymes (Arendsen et al., 2019). In bacteria, for instance, Cu^{+} and Cu^{2+} ions can interact electrostatically with peptidoglycan and other charged molecules of the cell wall. The ions undergo redox reactions at the bacterial cell surface, generating hydrogen peroxide (H_2O_2) to yield $OH^{\bullet}$ which can impair the stability of the plasma membrane. Inside the cells, copper cations can influence biochemical processes and displace metallic cofactors from binding sites in proteins, leading to their loss of function, or even bind to DNA forming double bonds and destabilizing the helical structure (Mukherji et al., 2012; Robinson et al., 2021).

One of the main suggested mechanisms for CuNPs/CuONPs antifungal action is based on cell wall disruption and intracellular toxicity after penetration. Fungal cell walls are composed mainly of a combination of glycoproteins and polysaccharides, i.e., glucan and chitin, as well as their complexes, with the basic function of protecting the cells against external stresses coming from the surrounding environment (Pinto et al., 2008). The toxicity starts with the binding of CuNPs/CuONPs on the cell wall, either by simple surface adsorption or other mechanisms, causing damage to the plasma membrane integrity. It is likely that a significant production of ROS occurs in close proximity to the membrane due to the presence of the nanoparticles, impairing the membrane dynamic equilibrium and eventually leading the fungal cell to apoptosis (Manke et al., 2013). Briefly, CuNPs and CuONPs are taken up by the cell through transport system, endocytosis and/or receptor-mediated uptake and release Cu^{2+} and Cu^{+} ions which diffuse into the cells that can interrupt mitochondrial respiratory chain and further generate additional ROS via Fenton-type and Haber-Weiss-type reactions. The CuNPs induce oxidative stress ($O_2^{\bullet-}$ and $OH^{\bullet}$) via Fenton-type reaction. CuNPs are further oxidized to CuONPs which reacts with H_2O_2 to induce $OH^{\bullet}$ via Haber-Weiss-type reaction and causes damage to nearby structures such as the mitochondria, lysosomes, endoplasmic reticulum, and ribosomes (Manke et al., 2013; Thannickal and Fanburg, 2000; Valko et al., 2006).

5.3 Copper nanoparticle-fungal cell interactions

The CuNPs can cause leakage of cellular electrolytes through disruption of cellular membrane impacted by an osmotic imbalance. As eluded above, CuNPs can release Cu^{2+} ions, besides generating an excess ROS that damage DNA and metalloproteins (Waldron and Robinson, 2009); Cu^{2+} ions are able to compete with other essential metals such as iron ions (Fe^{2+}) catalyzed by cuproenzyme, thus preventing proteins from binding with the correct cofactor and consequently inhibiting protein function (Robinson et al., 2021; Waldron and Robinson, 2009). Finally, those nanoparticles can agglomerate close to the nuclear membrane, sometimes entering the nucleus, causing damage to the genetic material and leading the cell to death (Safaei et al., 2019).

The Cu^{+} and Cu^{2+} ions released can chelate at the active sites of certain proteins affecting their pathways and stimulant for oxidizing stress. Ma and coworkers brilliantly elucidated the biomolecular mechanisms of CuNPs, specifically C-coordinated *O*-carboxymethyl chitosan-Cu complexes (O-CSLn-Cu) against *Phytophthora capsici* Leonian, an important plant pathogen for agriculture. Using the complexes at 150 ppm, the authors were able to identify a decreased hyphal viability and an intense impairment in cell membrane integrity after 12 h of the treatment. Morphological alterations in the mycelia were also observed, i.e., shrinking and swelling or sinking and swelling (Ma et al., 2020) probably attributed to Cu ROS-mediated cell death via mitochondrial dysfunction.

When it comes to the intracellular effects, the same authors identified 1172 proteins with altered expression after the treatment: about half of them were downregulated and the other half was upregulated. Those proteins could be grouped in three main categories regarding their function: biological process (BP), cellular component (CC), and molecular function (MF) proteins. Proteins pertaining to the BP group were involved in metabolic processes, cellular processes, localization and biological regulation, while the CC group comprised proteins and protein complexes present in the membrane and in important organelles. Finally, the MF group composed of proteins linked to catalysis, binding transporter activity, structural molecules, and antioxidant capacity (Ma et al., 2020). The biological processes with significant changes comprised the acetyl-CoA biosynthetic process (Acetyl-CoA is intrinsically involved in the catabolism and anabolism of nutrients such as carbohydrates, proteins, fatty acids, and nucleic acids); metabolism of glutathione, sulfur compounds, and acetate; enzymatic activities (CoA-ligase, acetate-CoA ligase, oxidoretuctase, and 5-methyltetrahydropteroyltri-L-glutamate-dependent methyltransferase); vesicles formation and transport (Ma et al., 2020).

Furthermore, several metabolites were differentially expressed due to O-CSLn-Cu action, located in different portions of the cell: 61 dispersed in the cytoplasm, 33 confined in the mitochondria, 16 in the plasma membrane, 7 in peroxisomes, 7 in adiposome, 7 in the Golgi apparatus, 10 in the peroxisome, and 7 in the myelin sheath. All those metabolites participate in 136 different cellular pathways, and the most important are the citrate cycle, protein digestion and absorption, and metabolism of purines, alanine, aspartate, and glutamate. This result corroborates with the previous assumption that CuNPs can exert important effects on the mitochondria since it was the most affected organelle (Ma et al., 2020). The confirmation of the significant involvement of mitochondria in the growth inhibition of *P. capsici* Leonian comes with the decreased activity of α-ketoglutarase dehydrogenase complex (α-KGDHC), as well as the decreased levels of ATP and citrate caused by the treatment with O-CSLn-Cu for 12 h.

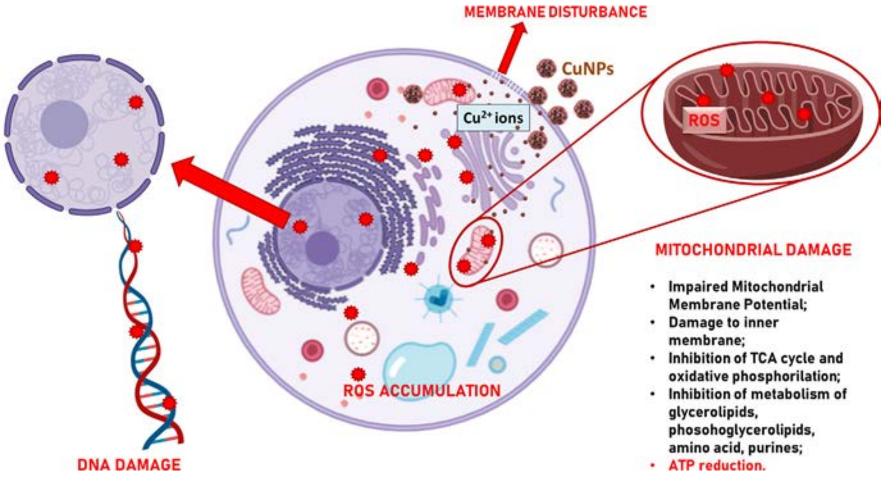

FIG. 3

Main mechanisms of CuNPs antimicrobial activity in eukaryotic cells, such as fungi: membrane disturbance, mitochondrial damage, ROS accumulation, and consequent damage to the DNA.

The low ATP levels might be further explained by a decreased citrate cycle yield, caused by the downregulation of citrate cis-aconitate, succinate, and L-malate metabolites. Furthermore, morphological alterations in those organelles could be observed, i.e., cristae disappearance and impairment of the bilayer conformation. Finally, the mitochondrial membrane potential (MMP) decreased significantly after the treatment. Mitochondria are not the sole responsible for the observed alterations, as the supplementation of ATP to normal levels was not enough to reverse the whole damage caused by the treatment (Ma et al., 2020). Once the damage to mitochondria is established, ROS can accumulate and react with important biomolecules, especially the DNA, leading the cells to death (Fig. 3). Indeed, an elevated level of ROS could be detected in *P. capsici* Leonian mycelia, corroborating the assumption that the damage to mitochondria plays a key role in the inhibition of the fungi (Fan et al., 2021; Hussain et al., 2021; Ma et al., 2020).

6 CuNPs and CuONPs ecotoxicology and safety

The increased use of nanoparticles in our daily lives even within the food chain has become the main concern to researchers and the general public, as there is still insignificant long-term data from chronic exposure and ecotoxicological profiles are still limited (Peixoto et al., 2020; Singh et al., 2021b). Humans are usually exposed to CuNPs through various indirect avenues such as dermal implants, ocular exposure, inhalation, and ingestion which can lead to their deposition in the upper and lower respiratory tract. It is imperative to comprehend CuNPs/CuONPs-plant interactions, including uptake, mobilization, and accumulation to fully understand the ecotoxicology profile of CuNPs/CuONPs destined for environmental applications such as in agriculture used as nanofungicides against pathogenic fungal contamination and can also serve as a trace micronutrient to enhance chlorophyll, seed production, and root health (Malandrakis et al., 2021; Singh et al., 2021b). The rigorous investigations are crucial in establishing regulatory frameworks assigned to nanoparticles such as CuNPs, and CuONPs. Currently, in some European Union (EU) countries, ionic Cu is permitted for use in both conventional and organic agriculture with regulatory limits up to a maximum dose between 1.5 and 6 kg/ha/year of elemental Cu depending on the type of crops. For example, the maximum dose limit for nuts (1.5 kg/ha/year); small fruits (2), and for a majority of crops (4 kg/ha/year) (Andrivon et al., 2018). The application of Cu varieties year-by-year is based on variations in disease-pressure years. However, some EU countries such as the Netherlands and Germany have prohibited the use of Cu as crop protection agents.

Mosa et al. (2018) investigated the phytotoxicity, genotoxicity of CuNPs on cucumber (*Cucumis sativus*) plants. Results revealed that CuNPs accumulated in the plant tissues with high amounts in the roots and also caused genomic alterations (copper-zinc superoxide dismutase (Cu-Zn SOD) gene expression) in *C. sativus* attributing kg/ha/year to the phenotypical changes of decreased biomass, photosynthetic pigments (Chlorophyll a and b), and root plasma membrane damage in a concentration-dependent manner. Their results can be explained by the function of Cu as a cofactor for many enzymatic activities, and its role in plant respiration and metabolism of carbohydrates and proteins during photosynthesis. It should be noted that the CuNPs used in this study were purchased with an average size of 20 nm and no other information was disclosed regarding how the CuNPs were synthesized and what capping/stabilizing agents were used during the production of CuNPs.

Maziero et al. (2020) highlighted the importance of species-specific in vitro and in vivo ecotoxicology evaluation of nanoparticles. They evaluated the ecotoxicological profile of gum arabic protein silver nanoparticles (AgNP-GP), and they pointed out that other factors, such as the type of reducing and stabilizing agent used during the synthesis in addition to the nanoparticle size, may also influence the overall toxicity profile. Cu can be found in nature as a metallic or as an ionic element (Cu^+ and Cu^{2+}). The use of CuNPs and CuONPs have been reported in several applications, this form of Cu has also been found in the environment. Although it is known that Cu is present in metabolic processes in humans and animals, at high concentrations and different conformations and or complexes, it may lead to toxicity. Whilst Cu is normally inert, in its ionic form it can bind with several organic substances interfering in environmental and physiological processes.

Song et al. (2014) reported that CuNPs demonstrated higher cytotoxicity against mammalian and piscine cell lines than ionic Cu^{2+}, indicating the species-specific toxicity of CuNPs. The authors emphasized the important role of the morphologies and size of CuNPs with the corresponding release of ionic Cu release attributing to the overall toxicity. Most recently, investigations of CuNPs, CuONPs, and Cu^{2+} on a variety of aquatic organisms such as zebrafish (*Danio rerio*), *Oncorhynchus mykiss*, *Apistogramma agassizi*, *Poecilia reticulata*, and many more revealed that particles <50 nm at concentrations ranging from 0.1 to 50 ppm affected intestinal development through induced ionoregulatory toxicity attributed to the decrease in H^+ pump and Na^+/K^+-ATPase activity. Other effects include induced hepatitis, nephrotoxicity, with chronic exposure reported to affect reproduction.

Work by Da Costa et al. (2020) evaluated the toxicity effects between bulk Cu and CuONPs (size = 37.5 ± 15.0 nm) facilitated by Cu^{2+} ions release and their effect on rice plants (*Oryza sativa*). Their inductively coupled plasma atomic emission spectroscopy (ICP-OES) measurements revealed that CuONPs and bulk Cu released ≤ 1 mg/L (0.63%) and ≤ 81 mg/L (7.79%) of Cu^{2+} ions, respectively. Treatment of the *O. sativa* showed that both CuONPs and bulk Cu caused a dose-dependent decline in CO_2 assimilation, plant growth, and photo-phosphorylation. Noticeably, the authors reported two different toxicity mechanisms; where bulk Cu was due to oxidative stress and CuONPs was caused by damage to thylakoid membrane due to NPs accumulation of nonionic form in chloroplasts without oxidative stress.

When the particle size of the CuNPs is large, the particles tend to accumulate in the lung tissue for a longer period leading to irritation and sensitization. On the other hand, when these particles are too small, they are able to move between cells or even permeate the cellular membrane or get into the blood circulation (Ameh and Sayes, 2019; Lee et al., 2016). Once in the blood circulation or the lymphatic systems, the nanoparticles may start to accumulate in other organs. This is especially worrisome for occupational workers in rubber and asphalt production industries who are extensively exposed to the emission of CuNPs. In the same way, physicochemical properties of nanoparticulated materials are what makes them very attractive to innumerous applications in the industry, these properties also have an influence on their facility in entering cells, translocating to organs and, therefore, in their toxicological behavior. There is no reference dose limit for chronic oral exposure or reference concentration for chronic inhalation exposure to CuNPs and CuONPs, which highlights the importance of developing studies and systematic methods to assess their toxicological levels. In the United States, the Environmental Protection Agency (EPA) has set maximum contaminant level for Cu is 1.3 ppm in drinking water (Ameh and Sayes, 2019; National Research Council, 2000).

Perrotta et al. (2020) investigated the consequence of the accumulation of engineered nanoparticles in freshwater using outdoor wetland mesocosm experiment for 6 months. They reported increased nutrient excretion rates of nitrogen and phosphorus which are associated with the growth and survival of consumers such as rooted macrophyte (*Egeria densa*), aquatic snails (*Physella acuta* and *Lymnaea* sp.), and eastern mosquito fish (*Gambusia holbrooki*). This was attributed to the chronic exposure due to low concentrations of Cu and AuNPs by altering the metabolism of consumers and increased consumer-mediated nutrient recycling rates as catalysts for a cascade of events that can potentially intensify eutrophication in aquatic systems.

The increase of utilizing CuNPs, CuONPs, and/or Cu nanoformulation complexes in agriculture as antifungal agents is mounting. It is imperative to clearly evaluate their life-cycle and establish regulatory limits, because when ingested via contaminated water or food (or even through breathing) at high concentrations; CuNPs has been reported to react with stomach acidic pH, approximately 84.5%

of CuNPs dissolved into Cu ions that can be rapidly absorbed, migrate through the intestines into the systemic circulation and to other organs (Ameh and Sayes, 2019; Lee et al., 2016). The prolonged exposure to airborne Cu particulates may cause diseases such as metal fume fever, impairment of gastrointestinal, respiratory, renal, and hepatic function. Some people are more sensitive to the toxicity of Cu due to genetic abnormalities, and also may manifest into Wilson's disease in adults, and in children, it can result in cirrhosis or idiopathic Cu toxicosis (Ameh and Sayes, 2019).

Adeyemi et al. (2020) studied the toxic effects of three commercially available nanomaterials: CuONPs, copper-iron oxide nanopowders, and carbon nanopowders. Usually, CuONPs are used as superconductors, sensors, or antimicrobial agents, while copper-iron oxide nanoparticles have been important in the biomedical area, for cancer targeted therapy, and as contrast agents. In vitro tests indicative of cytotoxic, genotoxic, mitochondrial disruption, and oxidative stress were carried out using human hepatoma HepG2 cell lines. The results revealed that the presence of the nanomaterials decreased the oxygen fluxes in cells when compared to controls. Moreover, elevated levels of superoxide anion, caused by the inhibition of respiration and, therefore, denoting mitochondrial dysfunction caused by the nanomaterials was reported. Results showed cytotoxicity, mutagenicity, oxidative stress, and mitochondrial impairment in cells with high concentrations of nanomaterials, in particular of CuONPs that showed to be extremely toxic.

Concerning environmental exposure, the main origin of CuNPs and CuONPs found in ecosystems comes from wastewater treatment efflux and agriculture. Some personal care products may contain CuNPs formulations and, after use, they go through wastewater into the water supply chain. In the agriculture field, CuNPs and CuONPs present in fertilizers and pesticides (Ameh and Sayes, 2019; Chibber and Shanker, 2017) can translocate into the soil, where they can be transported and transformed due to interactions with organic matters and other components within the soil and this can result in modified physicochemical properties which can lead to problem related to toxicity (Ameh and Sayes, 2019; Rasool et al., 2021). The toxicologic effects of nanoparticles are different depending on their physical properties, i.e., size, morphology, surface area, chemical composition, and solubility.

The smaller the nanoparticle provides a larger surface area considering a defined mass of material, and as a consequence, the surface reactivity with the surrounding medium (for instance, via dissolution and ion liberation) will be enhanced. The aggregation of CuNPs occurs in water according to the concentration of natural organic matter, pH, and ionic strength (Ameh and Sayes, 2019; Torres-Duarte et al., 2016). For the sedimentation process, the pH of the water and the size of the particle will be critical especially during the agricultural application of Cu nanofungicides. When Cu ions interact with other ions present in the water, changes in the capacity of CuNPs to suspend in water can be observed. Oxidation state, aggregation, pH, and natural organic matter will have an important role in the slow process of dilution of CuNPs. On the other hand, when CuNPs interact electrostatically with biopolymeric substances or even with the natural organic matter may induce stabilization and avoid particle aggregation and sedimentation (Ameh and Sayes, 2019). Table 2 shows the environmental interaction of CuNPs and CuONPs.

Table 2 Interaction of CuNPs with the environment.

Exposure medium	Species	CuNPs						
		Size (nm)	**Source**	**Conc.**	**Method**	**Objective**	**Effects**	**Results**
Terrestrial	Microorganisms (responsible for the degradation of pesticides)	40–60	Sigma-Aldrich	20 and 50 mg/L	Incubation of CuNPs and microorganisms in LB broth and mineral saline medium (MSM) media	Assess whether CuNPs in subinhibitory concentrations modify the frequency of conjugation (FC) of two conjugative catabolic plasmids (CCP)—pJP4 and pADP1	< CCP transfer; 10% reduction in FC of mating pairs; rapid release of Cu^{2+} ions (< 3 h) in a medium with a higher level of organic matter (LB)	Lower efficiency in pesticide degradation Parra et al. (2019)
	Cucumber (*Cucumis sativus*)	10–30	Hengqiu Graphene Technology (SUZHOU) Co. Ltd., Shanghai	50, 100, and 200 mg/L	Hoagland solution treatment with CuNPs in *C. sativs* for 4 days	Investigate the phytotoxicity of CuNPs in cucumber (*C. sativus*) plants hydroponically grown	Decrease in biomass by 100 and 200 mg/L; Cu accumulation in tissues with > evidence in the roots in proportion to concentration (aggregate form 80–140 nm); < chlorophyll content in proportion to concentration; > H_2O_2 and MDA levels; > H_2O_2 accumulation in the roots; > leakage of electrolytes to 50 and 200 mg/L; > SOD levels, relative gene expression of the Cu-Zn gene	Damage to the plasma membrane of the root and toxicity to the plant *C. sativus* Mosa et al. (2018)
	Wheat (*Triticum aestivum* L.)	19–47	Biosynthesis—native strain *Klebsiella pneumoniae*	25, 50, and 100 mg/kg	CuNPs were added to chromium (Cr)-rich soils (3.5 mg/kg $K_2Cr_2O_7$) for 30 days	Through biogenic CuNPs to evaluate wheat plants through Cr stress and their growth	For doses of 25 and 50 mg/kg: > root growth; > amount of biomass; > cellular antioxidant content; < H_2O_2 and MDA levels; < translocation of Cr from the soil to the roots and shoots; > amount of residual Cr in the soil, however < amount of Cr bioavailable in the soil	Cr immobilization in the soil, avoided translocation to the plant. Relieving cellular oxidative stress and contributing to healthy growth Noman et al. (2020)

Aquatic	Microorganisms (Anammox)	10–30	–	0.25–50 mg/g of suspended solid (SS)	CuNPs added to serum vials together with 10 mL of anammox biomass	Evaluate the impacts of CuNPs on anaerobic oxidation of ammonium in the sludge of granules and anammox flakes	1.25 mg/g of SS significantly inhibited the metabolic activity of anammox; 4.65 and 3.27 mg/g of SS caused 50% of the inhibition of the anammox metabolic activity for granules and flakes, respectively; 5 mg/g of SS caused severe accumulation of toxic N_2H_4 intermediate; concentrations; > 12 mg/g of SS limits the adsorption capacity of CuNPs and increases the amount of Cu^{2+} ions dissolved in water and the release of LDH	Smaller efficiency of the system anammox for the treatment of waste Zhang et al. (2017)
	Zebrafish embryos (*Danio rerio*)	25	IoLiTec, Inc. (Germany)	0.1–1 mg/L	CuNPs dispersed in egg water from 24 h	Evaluate innate immune responses of the zebra fish's dermal and intestinal system upon CuNPs exposure	For 1 mg/L: > CuNPs accumulation in the intestine and skin epithelium; alteration of chemokine *ccl20a*; increased neutrophils in the tail area; increased signal of IL1β and irg1l in the intestine and skin; reduction in the number of neuromats	Delay in embryo growth, cell death and cell extrusion of the epithelium Brun et al. (2018)

MDA, *malondialdehyde;* LDH, *lactate dehydrogenase;* IL1β, *interleukin 1 beta;* irg1l, *immunoresponsive gene 1-like.*

Taking into consideration the data presented in the studies presented in Table 2, one can notice that in certain concentrations, CuNPs can be somewhat harmful to the surrounding environment, leading to different effects and responses. However, the study by Noman et al. (2020) revealed that the interaction between CuNPs and the soil where wheat plants (*Triticum aestivum* L.) were growing generated good outcomes for the produce, compared to other studies. The authors affirm that these outcomes are due to the "green" origin and/or green production of the CuNPs. An important concern about the presence of CuNPs in the environment is the possibility of bioaccumulation in organisms, which can happen inside the cell membranes of single-cell microorganisms or in organs, systems of sea animals, and terrestrial plants. The investigations of the modifications these nanoparticles go through are paramount to understand their potential in bioaccumulation across food webs and ecotoxicity (Ameh and Sayes, 2019; Fischer et al., 2021; Singh et al., 2021b; Torres-Duarte et al., 2016).

7 Conclusion

The use of Cu nanocomplexes (i.e., CuNPs, CuONPs, and other formulations) as nanofungicides have been reported to exhibit superior antifungal activity against mycotoxigenic fungi. The use of these Cu nanocomplexes as nanofungicides provides an additional advantage over conventional fungicides, as CuNPs can be oxidized to form CuONPs, which can easily mix with polymers or macromolecules as vehicles that i7mprove their antimicrobial, chemical, physical, and mechanical properties. The CuNPs and CuONPs can also provide the chelated Cu as a micronutrient which is required in various pathways (cofactor for enzymatic reactions, metabolism, enhance chlorophyll, seed production, and root health) in plants. In addition, Cu is involved in the formation of collagen and elastin, assists in Fe absorption which enables the body to form red blood cells, and is crucial in energy production, and Cu biodistribution can be found in the brain, heart, liver, kidneys, and skeletal muscles which helps maintain healthy bones, blood vessels, nerves, and immune function. It is through this that sufficient and well-regulated amount of Cu can help prevent cardiovascular disease and osteoporosis.

It is important to consider the ecotoxicological profile of all nanoparticles used in agriculture as they become directly and indirectly with the food supply chain with a high possibility for chronic exposure to human and animal health. Cu has a numerous advantageous applications in agriculture attributed to its physiochemical factors (antimicrobial activity) and catalytic activity in water treatment which can be fed back into the agricultural system via irrigation. Additionally, Cu accumulation in soil and water body is prominent with constant usage as this can influence the toxicity of CuNPs, CuONPs, and other Cu complexes in aquatic and terrestrial organisms. However, the toxicity of the CuNPs as nanofungicides is dedicated by a number of factors such as the synthesis (reducing and/or capping agents utilized), concentrations, surrounding medium (often aqueous), dissolved organic substances/matter, pH, temperature, and salinity which are all ascendancy of Cu ionic (Cu^{2+} and Cu^{+}) dissolution, speciation, and toxicity, in addition to the type of crop or animal being evaluated for effectiveness against fungal and mycotoxin contamination.

Green nanotechnology has provided an avenue to limit the toxicity profile with enhanced catalytic activity of Cu nanocomplexes as nanofungicides and nanopesticides in agricultural production systems or the food industry that bring unprecedented impacts, especially in developing countries. Not only does it benefit from an economic point of view but also directly or even indirectly, in the improvement of the quality of life, increased food production per cultivated area, improving the quality of agro-industrial processes, and access to new products by a greater number of consumers. Several other sectors linked to agribusiness will inevitably benefit from green nanotechnology advances with the continuous incorporation of these new technologies. The main areas that will be significantly affected are the quality and certification of agricultural products, biotechnology, agro-energy, environmental monitoring, new uses of farm products, precision agriculture, and traceability, input industry (nanofertilizers, nanopesticides), innovations in veterinary drugs, and food preservation.

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Further reading

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